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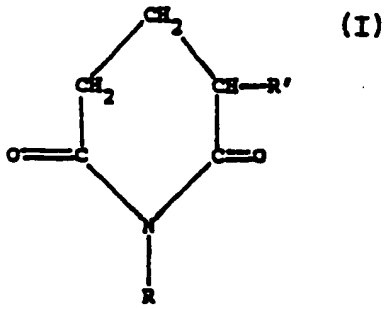

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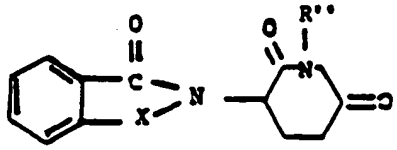

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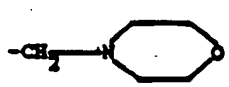

(a)

(57) Abstract

Compounds of structure (I), wherein R is selected from the group consisting of hydrogen, alkyl radicals of 1-6 carbon atoms, the phenyl radical, and the benzyl radical; and wherein R' is selected from the group consisting of the phthalimido radical and the succinimido radical and f structure (II), wherein X is CH_2 or C=O; R'' is H, $-CH_2CH_3$, $-C_6H_5$, $-CH_2C_6H_5$, $-CH_2CH=CH_2$, or (a) and hydrolysis products of said compounds wherein R'' is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed are useful for the control of abnormal concentrations of TNF without substantially effecting the concnetration of ther cytokines.

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"METHOD FOR CONTROLLING ABNORMAL CONCENTRATION TNFα IN HUMAN TISSUES"

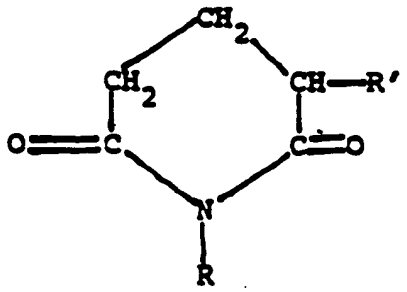
RELATED APPLICATIONS

This application is a continuation-in-part of copending application serial number 07/655,087 filed February 14, 1991.

BACKGROUND OF THE INVENTION

Debilitation, i.e. loss of weight, strength, vascular weakness, and other symptoms are natural sequelae of many diseases which afflict humans. These may include, for example bacterial infections such as tuberculosis; viral infections, particularly retroviral infections including HIV infections such as AIDS; various forms of arthritis particularly rheumatoid and degenerative; ulcerative colitis; regional enteritis; and the like. Human patients with these symptoms may present with an acute condition such as septic shock or with a chronic condition such as cachexia.

United States Patent 2,830,991 describes a class of therapeutic agents of the general formula I



I

wherein R is selected from the group consisting of hydrogen, alkyl radicals containing 1-6 carbon atoms, the phenyl radical, and the benzyl radical; and wherein R′ is selected from the group consisting of the phthalimid radical and the succinimid radical.

The subject matter of this patent and of any other patents or publications identified in this disclosure are incorporated herein by reference.

Preferred compounds within the scope of the above formula I, for use in this invention are:

3-phthalimido-2,6-dioxo-1-ethyl piperidine
3-phthalimido-2,6-dioxo-1-phenyl piperidine
3-phthalimido-2,6-dioxo-1-benzyl piperidine
3-phthalimido-2,6-dioxo-1-allyl piperidine
3-phthalimido-2,6-dioxo-piperidine

As described in the patent, the compounds are produced by reacting an aliphatic dicarboxylic acid, which contains five carbon atoms in a straight chain, the methylene groups of which are substituted by the substituents in accordance with the appropriate general formula, with urea or substitution products thereof or with a primary amine or an acid amide in such manner that water is split off and the ring is closed. If an amino group is present in the aliphatic chain, this group must not exist in free form in this stage of the process, since otherwise there is the danger of this amino group participating in an undesirable manner in the reaction. Instead of using the dicarboxylic acid, it is also possible to employ functional derivatives thereof, such as acid halides, acid esters and acid amides.

Compounds of the glutaminic acid series may be used as starting materials f r the present invention. In this case als , th acid halides, esters and amides of glutaminic acid may be empl yed instead of th acid itself. It is kn wn that glutaminic acids tends to form

5-membered rings with a free amino group. This reaction is undesirable for the purposes of the present invention. The amino group must therefore be substituted or protected prior to the ring-closing reaction. The protection of the amino group may be carried out, when using products of the glutaminic acid series, by introducing the phthalyl, succinyl or like radical in a manner known per se. The proportions of the components used for the ring formation must be such that at least 1 mol of the compound yielding the imide nitrogen is used to one mol of the glutaminic acid component.

The first compound listed above is prepared by reacting 27.7 g. of N-phthalyl glutaminic acid with 66 g. of a 33% solution of ethyl amine in water and slowly heating in an oil bath 160-180°C., the mixture being maintained at this temperature for 15 to 20 minutes. The reaction product is recrystallised from alcohol by fractionation. It melts at 209°C.

The last compound listed above prepared by reacting 13 g. of phthalyl glutaminic acid anhydride and 6 g. of urea in 75 cc. of absolute xylene for 4 hours at the boiling point of the mixture. Formation of a sublimate takes place with evolution of ammonia and carbon dioxide. The xylene is then distilled off in vacuo and the residue recrystallized from 95% alcohol by fractionation. In addition to some phthalimide and phthalyl glutamine, the required N_2-phthalyl glutaminic acid imide is obtained, having a melting point of 269-271 C.

In the patent, the compounds are disclosed as having low toxicity and as useful for certain spasmolytic and antihistaminic effects. The compound 3-phthalimido-2,6-dioxopiperidine is disclosed as being particularly useful as a sedative. This compound was marketed as a sedative under the generic name thalidomide. It was subsequently discovered to be teratogenic and was withdrawn from the market.

Despite its teratogenicity, thalidomide has long been employed for the treatment of erythema nodosum leprosum (ENL) an accute inflammatory state occurring in lepromatous leprosy. See, for example Mellin, G.W., and M. Katzenstein. N. Engl. J. Med. 267:1184 (1962). More recently, it has been shown to be useful in the treatment of graft-versus-host disease by Vogelsany, G.B., S. Taylor, G. Gordon and A.D. Hess. Transplant Proc. 23:904 (1986); for treatment of reheumatoid arthritis by O. Gutierrez-Rodriguez, P. Starusta-Bacal and O. Gutierrez-Montes. The Journal of Rheumatology 16:2 158 (1989); and for treatment of aphthous ulceration in patients positive for HIV antibody. Brit. Med. J. 298:432 (1989).

The tumor necrosis factor (TNF-α) is one of several cytokines released mainly by mononulear phagocytes together with several other cytokines in response to stimuli to the immune system. It is required for a cell mediated immune response to overcome infections. As its name suggests, it is associated with the destruction of tumor cells. It is not present in measureable amounts in normal sera, but appears, often very rapidly, in response to immune stimulators such as bacterial and viral infections, particularly HIV infections. In the case of chronic infection it may b

found in the sera at relativ ly high or low l vels for extend d p riods of tim . It may also app ar suddenly in high concentrations in response to release of a toxin by an invading bacteria. It is markedly elevated in ENL.

TNF-α has been recognized as manifesting a dose dependent toxicity. If present at low levels for too long a period it results in cachexia. At high levels even for a short time it results in septic shock.

Cachexia is a general weight loss and wasting occurring in the course of a chronic disease. More specifically, it is a weight loss not accounted for by decreased caloric intake. It is associated with cancer, the opportunistic infections of AIDS, inflammatory diseases, parasitic diseases, tuberculosis, high dose IL-2 therapy and the like. It is a chronic condition related to chronic diseases.

Septic shock is an acute condition usually, but not always attributed to infection or to toxic substances in the tissue. It is characterized by hypotension due to loss of vascular tone. It may result in patient collapse, or even death if not treated promptly and efficiently.

The retroviruses are a broad group of RNA viruses which, during their replication, employ the reverse transcription enzyme (RT) to convert a RNA message to DNA. The retroviridae family of viruses includes lentiviruses (visna, maedi, progressive pneumonia virus - "sl w viruses"), spumaviruses (f amy viruses) and

onc rnavirus s (types A, B, C, D, RNA tum r viruses). The retroviruses have been shown to infect murine, avian, feline, primate, and human species.

The human immunodeficiency virus (HIV-1) or human T-Cell lymphotropic viruse (HTLV-III) which causes Acquired Immune Deficiency Syndrome (AIDS), AIDS related complex (ARC) and other AIDS related diseases is a retrovirus. TNF-α functions in an autocrine manner in the induction of HIV-1 expression (G. Poli et al, PNAs Vol 87 p 782, 1990).

It is apparent, therefore, that it is necessary to control the concentration of TNF-α in the sera to avoid the debilitating effects of abnormal concentrations of this cytokine including, for example, cachexia and septic shock.

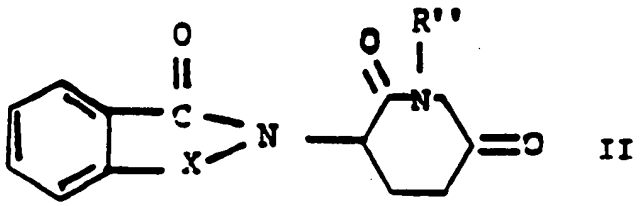
Other cytokines which are necessary for a proper immune response are also produced by mononuclear phagocytes. These include, for example, various interleukins such as IL-1 , IL-6, IL-8 and the granulocyte macrophage colony stimulating factor, GM-CSF. Still other cytokines are produced by the T-cells. It is desirable to control the concentration of TNF- without appreciably affecting the concentration and activity of other cytokines.

Heretofore, antiinflammatory and immunosuppresive steroids such as prednisolone and dexamethasone have been employed to treat the debilitating effects of TNF-α . Unf rtunately, thes therapeutic agents als block the producti n f other cyt kin s s that the patients b c me susceptibl t life threatening infecti ns.

BRIEF SUMMARY OF THE INVENTION

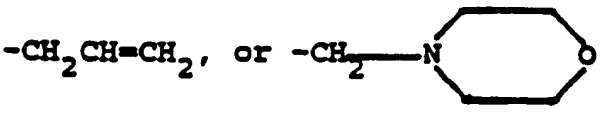
It has now been discovered that the debilitating effects of toxic concentrations of TNF-α, whether acute or chronic, can be controlled in humans by treating a human patient in need of such treatment with an anti-debilitating amount of a compound within the scope of the above description. Typically the treatment may be either oral or parenteral, for example intravenously or subcutaneously.

It has further been discovered that certain compounds within the scope of the above formula as well as other closely related compounds are especially useful for the practice of this invention. These compounds are presently preferred for the therapeutic purposes of the inventions. These preferred compounds include those represented by formula II



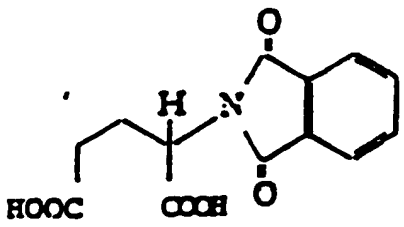
II

wherein X is CH_2 or C=O; R is H, $-CH_2CH_3$, $-C_6H_5$, $-CH_2C_6H_5$,



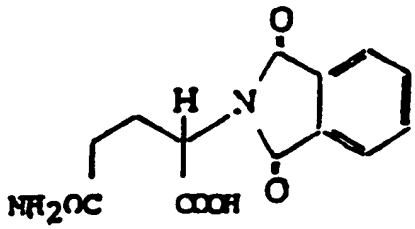
and hydrolysis products of said compounds wherein R is H and the peperidine ring or both the peperidine and the imido ring are hydrolyzed.

Especially preferred compounds within the ambit of the above definition are represented by the formulas:



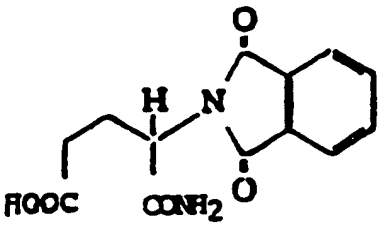
N-phthalyl-*D,L*-glutamic acid

A



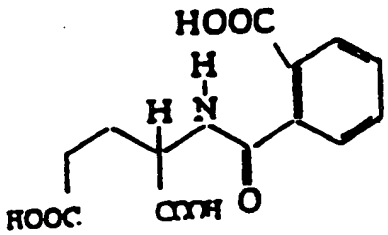
N-phthalyl-*D,L*-glutamine

B



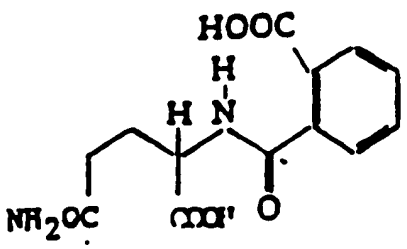
N-phthalyl-*D,L*-isoglutamine

C



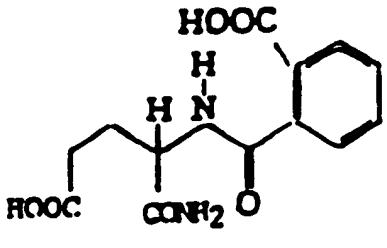
N-[carboxybenzoyl]-*D,L*-glutamic acid

D



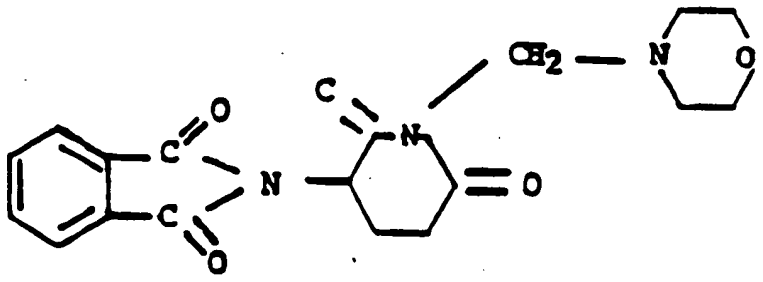
N-[o-carboxybenzoyl]-
D,L-glutamine

E



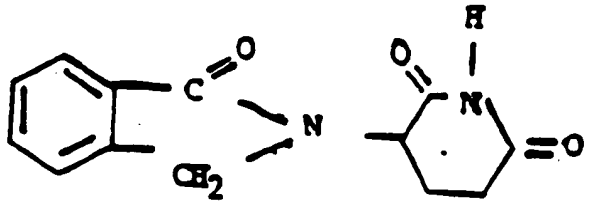
N-[o-carboxybenzoyl]-
D,L-isoglutamine

F



G

1-Morpholino methyl-3-phthalimido-2,6-dioxo piperidine



H

3-phthalimidino-2,6-dioxo piperidine

Most of the non-hydrolyzed compounds whose formulas are given above can be prepared by the processes described in the aforesaid U.S. Patent 2,830,991. The preparation of the phthalimidine compounds is described in U.S. Patent 3,705,162. U.S. Patent 3,563,986 describes the preparation of the morpholino substituted compounds. The hydrolytic compounds are prepared by standard hydrolysis procedures several of which will be known to the skilled artisan.

Those skilled in the art will recognize that the compounds of the invention exist in different recemic modifications. The racemic mixtures of the separate isomers are included within the scope of the invention.

The compounds may be administered alone, but will normally be employed in a composition containing a pharmaceutically acceptable carrier. It may be advantageous, as will be discussed more fully below to administer the selected compound or compounds together with an effective amount of a therapeutic agent appropriate for treating the cause of the abnormal concentration of TNF-α, for example with an antibacterial agent if the condition under treatment is shock caused by the sudden release of large amounts of a toxin because of bacterial infection.

THE DRAWINGS

Figs. 1, 2, 3, show the effects of thalidomide on TNF-α production in the presence of various reagents.

Figs. 4 through 7 show the results of studies conducted to stablish the utility of th comp unds of this invention to inhibit HIV-1 RT activity.

The drawings and the balance of this disclosure will be better understood by recognizing the meanings of certain abbreviations. CWP-ML means cell wall protein of Mycobacterium leprae. ENL means erythema nodosum leprosum. GM-CSF means granulocyte macrophage colony - stimulating factor. PPD means purified protein derivative of tuberculin. PBMC means peripheral blood mononuclear cells.

The studies described hereinafter will be recognized by those skilled in the art as establishing that the compounds of this invention selectively inhibit the production of human TNF-α without substantially affecting the production of other proteins or of total serum protein. Therefore, although the compounds of the invention will not cure diseases, they will significantly improve the quality of life of the patients. An important consequence of the study is the finding that TNF-α secretion is not totally inhibited. This is important since, as indicated above, TNF-α appears to be an essential mediator in the immune response.

There follows a complete description of one procedure for establishing the ability of the compounds of this invention to inhibit the production of TNF-α without inhibiting the production of other cytokines.

Mon cyte Isolati n. PBMC obtain d by Fic ll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density centrifugati n were rosetted with

neuraminidase-treated (Vibrio cholerae neuraminidase; Calbiochem-Behring Corp. La Jolla, CA) sheep erythrocytes (Scott Laboratories, Friskville, RI) (SRBC rosetting), and the nonrosetted cells were counted (E^- population monocytes enriched). 10^6 cells were cultured at 37°C in 24-well plates (Corning Glass Works, Corning, NY) in 1 ml of RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% AB^+ serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM l-glutamine. Adherent E^- cells were used for the studies.

Cytokine Agonists. LPS of Salmonella minnesota R595 (List Biological Laboratories, Campbell, CA) was diluted in PBS, pH 7.4, and used at 1 g/ml; Purified protein derivative of tuberculin (PPD) was purchased from Statens Seruminstitut, Copenhagen, Denmark; CWP-ML was prepared using known and published methods. The concentrations of the stimulating agents were those known to induce optimal TNF-α protein production by cultured monocytes. The endotoxin content of solutions and mycobacterial preparations was estimated by the Limulus amebocyte lysate assay (LAL; Whittaker M.A. Bioproducts, Walkersville, MD). All solutions used contained less than 10 pg/ml of endotoxin.

Cytokine Induction. Adherent E^- cells were stimulated with 1 µg/ml of LPS, 10 µg/ml of PPD, or 10 µg/ml of CWP-ML for up to 18-20 h. At various times, supernatants were harvested, centrifuged to remove cells and debris, and kept frozen until use (-20°C).

TNF-α Assay. TNF-α concentration in the supernatants was determined with a TNF-α specific ELISA, specific for the biologically active molecule. Assays were performed in 96-well plates (Nunc

Immun plates, Roskild , Denmark) coat d with th affinity-purifi d rabbit anti-TNF- α antib dy (0.5 μg/ml; 12-16 h; 4°C) and blocked for 2 h at room temperature with PBS/0.05% Tween 20 (Sigma Checmial Co., St. Louis, MO) containing 5 mg/ml BSA. After washing, 100 μl of TNF-α standards, samples, and controls were applied to the wells, and the plates were incubated for 12-24 h at 4°C. After the incubation, plates were washed and a second antibody, horseradish peroxidase (HRP)-conjugated mouse monoclonal anti-TNF-α , diluted 1:2,000 in PBS/BSA/Tween, was applied to the wells and incubated for 2 h at room temperature. The color reaction was developed with the OPD substrate (0.4 mg/ml o-phenylenediamine [Sigma Chemical Co.] in 24 mM citric acid, 51 mM sodium phosphate, pH 5.0 [phosphate-citrate buffer; Sigma Chemical Co.] containing 0.012% hydrogen peroxide [H_2O_2; Fisher Scientific Co., Pittsburgh, PA]) and absorbance read at 492 nm in an automated ELISA reader (Dynatech Laboratories, Inc., Alexandria, VA).

IL-1 Assays. IL-1 levels were determined using a commercial ELISA kit (Cistron Biotechnology, Pine Brook, NJ) according to the manufacturer's specifications. IL-1 levels are expressed as pico-grams per milliliter of protein.

IL-6 Assay. IL-6 levels were determined using a biological assay as described by Finkelman et al. Proc. Natl. Acad. Sci. USA. 83:9675 (1986). Proliferation of 7TD1 hybridoma cell line specifically sensitive to IL-6 was measured by colorimetric determination of hexosaminidas levels, Laudegren t al J. Immun l. M th ds. 67:379 (1984), and valu s for IL-6 in the

samples were obtained by interp lation from a standard curve. 1 U/ml of IL-6 corresponds to the concentration that yields half-maximal growth.

Granulocyte/Macrophage CSF GM-CSF Assay. GM-CSF levels were determined using a commercial ELISA kit (Genzyme, Boston, MA) according to the manufacturer's specifications, and were expressed as picograms per milliliter of protein.

Thalidomide Inhibition. The thalidomide used in this study was the purified drug (racemic mixture: D[+] and L [-] forms) (lot No. JB-I-114; Andrulis Research Corporation, Beltsville, MD). The compound was shown to be at least 99% pure, as analyzed by Fourier Transform Infrared Spectrum. It was then diluted in DMSO (Sigma Chemical Co.); further dilutions were done in sterile PBS.

Percentage inhibition of TNF-α secretion was calculated as: 100 x [1-(TNF-α experimental/TNF-α control)]; where TNF-α experimental represents TNF-α secretion by stimulated monocytes that were cultured in the presence of thalidomide, and TNF-α control represents TNF-α secretion by stimulated monocytes that were cultured in the absence of the drug. Monocytes cultured in medium containing equivalent amounts of DMSO in the presence or absence of the stimulating agent were used as controls for thalidomide-treated cells. Neither thalidomide nor DMSO had any effect on cell viability or function at the concentrations used.

Protein Synthesis. Human m n cytes w r cultur d in Tefl n beakers in methi nine-free RPMI with 10% AB^+ serum at 37 C f r 1 h, when 200 uCi/ml ^{35}S-methionin

(1,153 μCi/mm l; ICN Bi medicals Inc., CA) was add d to the cultur s f r th n xt 3 h with r with ut th stimulating and the suppressive agent. At the end of the labeling period, ^{35}S-labeled cells were washed twice in ice-cold PBS and lysed directly in 500 μl lysis solution (10 mM Tris-HCl buffer, pH 7.4 150 NaCl, 1 mM EDTA, and 1% SDS). Resolving 8% SDS-PAGE was performed overnight. The gel was washed, dried, and analyzed by autoradiography at -70°C using XAR-5 radiographic film (Kodak, Rochester, NY) with an intensifying screen.

RESULTS OF THIS STUDY

Monocytes were enriched from PBMC of normal donors and stimulated in vitro for 18-20 h with bacterial LPS and mycobacterial products, known agonists of monocyte TNF-α synthesis and secretion. Thalidomide suppressed LPS-stimulated TNF-α production (Fig. 1A) with a 50% inhibitory concentration (IC_{50}) of 1-4 μg/ml, and 90% inhibition observed at 10 μg/ml (18-20-h assay). Similar results were obtained when PPD and CWP-ML were used as stimulants (Fig. 1, B and C, respectively).

Fig. 1 shows the effect of thalidomide on (A) bacterial endotoxin (LPS, 1 μg/ml), (B) PPD, (10 μg/ml), and (C) CWP-ML (10 μg/ml)- induced TNF-α production. Monocytes were stimultaneously incubated with 2ng/ml to 10 μg/ml of thalidomide in the culture medium. Control cells were cultrued in medium alone. A dose-dependent inhibition of TNF-α secretion by thalidomide is apparent. No detectable production of TNF-α protein was bserved in supernatants f unstimulated m nocytes. Data represent m an ± SD f 15(A), tw (B), and one (C) different exp riments, respectively.

The inhibition of TNF-α secretion by thalidomid was dependent upon the state of monocyte stimulation as shown in Table 1. Preincubation of unstimulated monocytes with thalidomide, followed by removal of the drug before LPS stimulation, did not lead to suppression. By comparison, when LPS and thalidomide were added stimultaneously to the cultures, irreversible suppression occurred, even when the drug was removed after a few hours (Table 1). Therefore, the thalidomide sensitive reaction(s) occurs only after the LPS induction of TNF-α production.

TABLE 1

	h			h			
A	0-4	0	0	4-20	0	+	100
B	0-4	+	0	4-20	0	+	90 ± 4.6
C	None	0	0	0-4	+	+	48 ± 15
D	0-4	+	+	4-20	0	+	56 ± 0.5
E	None	0	0	0-20	+	+	52 ± 9.3

Human monocytes cultured in 24-well plates were preincubated with the inhibitory drug with or without the stimulating agent. After 4 h, the cultures were washed, medium was replaced, and LPS was added again for the next 16 h. Culture supernatants were recovered at the different periods and TNF-α levels determined as described. LPS-inducted release of TNF-α by monocytes cultured for 20 h in the absence of thalidomide (A). No inhibitory action of thalidomide was detected when th

drug was washed away before the addition of the stimulating agent (B). Thalidomid -induced inhibiti n of TNF- production in the present of LPS after 4 h of stimulation (C), which persisted even after the drug was washed away (D). Control experiment in which thalidomide was kept in the cultures with the stimulating agent during the whole assay (E). Data represent mean ± SD of two different experiments.

The inhibition of LPS-stimulated TNF-α secretion by thalidomide occurs in a setting in which many other proteins are being synthesized by both constitutive and induced mechanisms. Thus, a simple explanation for the effect of the drug on TNF-α production could be a suppression of overall protein synthesis.

Fig. 2 illustrates the effect of thalidomide on the pattern and quantity of proteins synthesized after a 3-h pulse of ^{35}S-methionine. The total incorporation of isotope into TCA-precipitable proteins as well as the intensity of most of the individual bands on SDS-PAGE of LPS-triggered monocytes remained unchanged after thalidomide treatment.

In Fig. 2 can be seen the effect of thalidomide on protein synthesis by human peripheral blood monocytes. Electrophoretic analysis of lysates from monocytes incubated with ^{35}S-methionine was performed. Cells were stimulated <u>in vitro</u> with and without LPS in the presence or absence of thalidomide at 1 and 4 µg/ml. TCA-precipitable radioactivity (10% TCA precipitation) was measured by liquid scintillati n c unting. Th amount f radioactivity in the p llets expressed as cpm x 10^{-3} and repr sents the mean of three precipitates with a SD of 10%. Neither total radi activity nor th pattern of

most of the protein bands in the gel was affected by thalidomide (lane 1) unstimulated cells, 3.3 x 10^{-2} cpm in TCA precipitates; (lane 2) cells stimulated with 1 µg/ml LPS, 4.2 x 10^{-2} cpm in TCA precipitate; (lane 3) cells stimulated with LPS in the presence of 1 µg/ml thalidomide, 4.2 x 10^{-2} cmp in TCA precipitate; (lane 4) cells stimulated with LPS in the presence of µg/ml thalidomide, 4.1 x 10^{-2} cpm in TCA precipitate; (lanes 5 and 6) cells incubated only with thalidomide at 1 or 4 µg/ml, respectively, 3.2 x 10^{-2} and 2.8 x 10^{-2} cpm in TCA precipitates, respectively.

Several cytokines are produced by monocytes in response to LPS in addition to TNF-α , including IL-1 and IL-6. Fig. 3 shows that thalidomide exerts a selective effect by suppressing only TNF- α secretion by LPS-stimulated monocytes. Whereas 4 µg/ml thalidomide suppressed TNF-α production (41.9% inhibition) (Fig. 3A), neither IL-1 (Fig. 3B), IL-6 (Fig. 3 C), nor GM-CSF production (Fig. 3 D) was influenced by the drug. Similar but more extensive selective suppression was observed with much higher (up to 20 µg/ml) concentrations of thalidomide. It was also observed that the D (+) enantiomer appeared to be more active than the L(-) enantiomer.

Fig. 3 shows the levels of different cytokines tested in culture supernatants of human monocytes stimulated with LPS for 6 h (A-C) or 20 h (D) in the presence or absence of 4 or 10 µg/ml of thalidomide. Data represent mean ± SD of six different experiments for TNF-α and IL-1 determinations and three experiments for IL-6 and GM-CSF measurements. About 41.9 ± 14.6% and 52.8 ± 14.7% inhibition of TNF-α secretion was found in the presence of 4 and 10 µg/ml of

thalid mid , r sp ctiv ly. "C nt" illustrat s unstimulated cells cultured in medium. No effect on IL-1 , IL-6, or GM-CSF secretion was detected in these cultures.

The following study establishes the utility of compounds of the invention for reducing TNF-α concentration in HIV infections. TNF-α is known to induce HIV replication. Similarly, it is known that peripheral blood monocytes from HIV infected patients secrete higher amounts of TNF-α than do monocytes from uninfected individuals. TNF-α is a cytokine capable of inducing viral expression in cells chronically infected with HIV. The art, therefore, has long been concerned with discovering products capable of inhibiting TNF-α production in HIV infected patients. The compounds of this invention are capable of so doing. This fact was established in studies using the known and commercially available chronically infected cell lines U1 and ACH-2, a promonocytic cell line and a T-lymphocytic cell line. The procedure employed is described by Poli et al. (1990) Proc. Nat'1. Acad. sci. U.S.A. Vol. 87, pp 782-785.

Briefly, the expression of HIV was upregulated by the addition of 10^{-7}m of phorbol 12-myristate 13-acetate (PMA) or 1 μg/ml of TNF-α to ACH-2 and U1 cells. The cells were suspended at $4X10^5$ per ml in RMPI 1640 medium (M.A. Bioproducts) supplemented with 10% (vol/vol) fetal calf serum in the presence of the selected amount of stimulator at 37°C in 5% CO_2/95% air for 48 hours, the sup rnatants c ll cted and test d f r the presence of Mg^{++} d pendent reverse transcriptas activity using the procedure f Willy et al (1988) J. Vir l. 62, 139-147.

For the test, 10 µl of supernatants were added to 50 µl of a mixture containing 5 µg per ml of poly(rA) p(dT) 12-18, (Pharmacia), 5mM $MgCl_2$ and 10 µci/µl of ^{32}P-labeled deoxythymidine 5' - triphosphate (dTTP-Amersham), and the mixture was incubated for 1 1/2 hours at 37°C. Eight microliters of the mixture were spotted onto DE81 paper (Whatman), air-dried and washed 5 times in 2X standard saline citrate buffer, and two additional times with 95% ethanol. The paper was dried, cut and radioactivity assayed. The results are shown in the figures.

Fig. 4 shows the results of tests in which 5, 10 and 50 µg/ml of thalidomide (THAL) and the known TNF-α inhibitor pentoxyfylline (PTN) were used to inhibit reverse transcriptase production with the cell line U1. For the comparison, reverse transcriptase activity in the absence of the inhibitor was taken as 100%. It will be seen that at a concentration of 50 µg/ml, thalidomide was as effective as PTN.

Fig. 5 shows the results of a similar test with a U1 cell line stimulated with PMA comparing thalidomide and PTN with other compounds of the invention including the D isomer of thalidomide. The other compounds of the invention are identified in this and the following figures by the letters used under their formulas hereinabove.

Fig. 6 shows a similar study in which the same compounds were tested with ACH-2 stimulated with TNF-α.

Fig. 7 records the results of a test using th ACH-2 cell line stimulated with PMA.

The compounds of the invention or their pharmaceutically acceptable salts may be administered perorally in a pharmaceutical carrier in standard form such as tablets, pills, lozenges, dragees and similar shaped and/or compressed preparations. It is also possible to produce emulsions or suspensions of the compounds in water or aqueous media such as unsweetened fruit juices and by means of suitable emulsifying or dispersing agents. They may also be employed in the form of powders filled into gelatin capsules or the like.

Such powders and mixtures for use in the preparation of tablets and other shaped and/or compressed preparations may be diluted by mixing and milling with a solid pulverulent extending agent to the desired degree or firmness or by impregnating the already milled, finely powdered, solid carrier with a suspension of the compounds in water or with a solution thereof in an organic solvent and then removing the water or solvent.

When preparing tablets, pills, dragees, and the like shaped and/or compressed preparations, the commonly used diluting, binding, and disintegrating agents, lubricants, and other tableting adjuvants are employed, provided they are compatible with agent to be administered. Such diluting agents and other excipients are, for instance, sugar, lactose, levulose, starch, bolus alba; as disintegrating and binding agents, gelatin, gum arabic, yeast extract, agar, tragacanth, methyl cellulose, pectin; and as lubricants stearic acid, talc, magnesium stearate, and thers.

They may be administered in the form of suppositories, typically utilizing such commonly used suppository vehicles, as cocoa butter.

The compounds may also be administered parenterally employing aqueous solutions or suspensions of water-soluble compounds or suspensions. The compositions may be made isotonic e.g. with salt or other solute and may contain a buffer, for example a phosphate buffer.

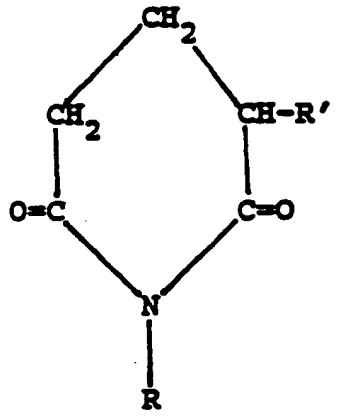
As indicated above, the compound employed in the invention may be the only active ingredient administered or it may be coadministered with another therapeutic agent in an amount which is effective to treat the condition associated with the debilitating effect. For example, if the cause of the condition is a toxin released by an infectious bacteria, an antibiotic such as tetracycline, penicillin, streptomycin and the like may be coadministered. If there is hypotension associated with lack of vascular tone, a vasopressive agent such as epinephrine or dopamine may be coadministered. If the patient is under treatment with a chemotherapeutic agent such as adriamycin, the compound of the invention and the chemotherapeutic agent may be coadministered.

The term "coadministered" does not mean that the compound of the invention and the additional therapeutic agent are administered in the same dosage unit, although they may be so administered. It means that they are administered within the same time span.

An "effective amount" of the compound or additional therapeutic agent will vary with the condition being treated, the age, weight and general physical condition of the patient under treatment and other factors readily evaluated by the physician in attendance.

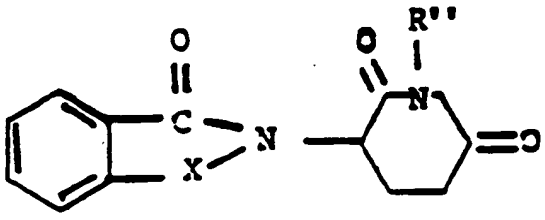
WHAT IS CLAIMED IS:

1. A method of treating the debilitating effects of toxic concentrations of TNF-α in a human patient in need of such treatment which comprises administration of an anti-deblitating amount of a compound of the formula I:

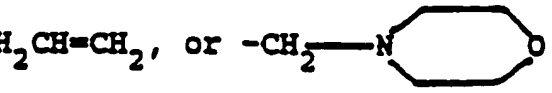


wherein R is selected from the group consisting of hydrogen, alkyl groups containing 1 to 6 carbon atoms, phenyl and benzyl; and R′ is selected from the group consisting of the phthalimide radical and the succinimido radical.

2. A method of treating the debilitating effects of toxic concentrations of TNF-α in a human patient in need of such treatment which comprises administration of an anti-deblitating amount of a compound of the formula II:



wherein X is CH_2 or C=O; R'' is H, $-CH_2CH_3$, $-C_6H_5$, $-CH_2C_6H_5$,

$-CH_2CH=CH_2$, or 

and hydrolysis products of said compounds wherein R'' is H and the piperidino ring or both the piperidino and the imido ring are hydrolyzed.

3. A method as in claim 1 or 2 wherein the debilitating effect is septic shock.

4. A method as in claim 1 or 2 wherein the debilitating effect is cachexia.

5. A method as in claim 1 or 2 wherein the debilitating effect is a result of an HIV infection.

6. A method as in claims 1, 2, 3, 4 or 5 wherein the compound is 3-phthalimido-2,6-dioxo-piperidine.

7. A method as in claims 1, 2, 3, 4 or 5wherein the compound is 1-morpholino methyl-3-phthalimido-2,6-dioxo-piperidine.

8. A method as in claims 1, 2, 3, 4 or 5 wherein the compound is 4-phthalimidino-2,6-dioxo-piperidine.

9. A method as in claims 1, 2, 3, 4 or 5 wherein the compound is N-phthalyl-isoglutamine.

10. A method as in claims 1, 2, 3, 4 or 5 wherein the compound is N-[carboxybenzyl]glutamic acid.

11. A method as in claim 1, 2, 3, 4 or 5 wherein the compound is coadministered with an amount of another therapeutic agent which is effective for treating the disease associated with the debilitating effect.

12. A method as in 11 wherein the compound 3-phthalimido-2,6-dioxo-piperidine.

13. A method as in claim 11 wherein the compound 1-morpholino methyl-3-phthalimido-2,6-dioxo-piperidine.

14. A method as in claim 11 wherein the compound 4-phthalimidino-2,6-dioxo-piperidine.

15. A method as in claim 11 wherein the compound N-phthalyl-isoglutamine.

16. A method as in claim 11 wherein the compound is N-[carboxybenzoyl]glutamic acid.

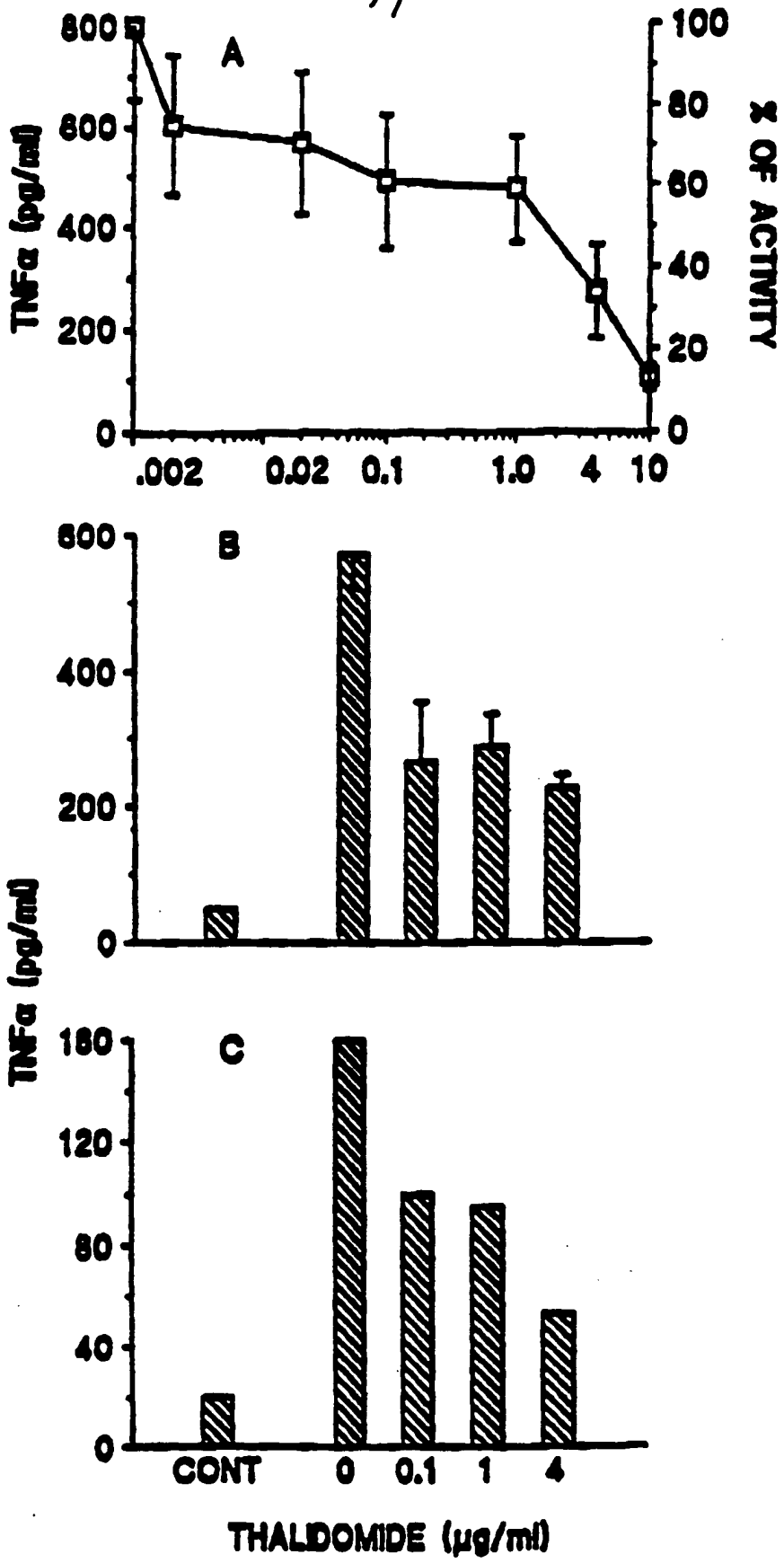
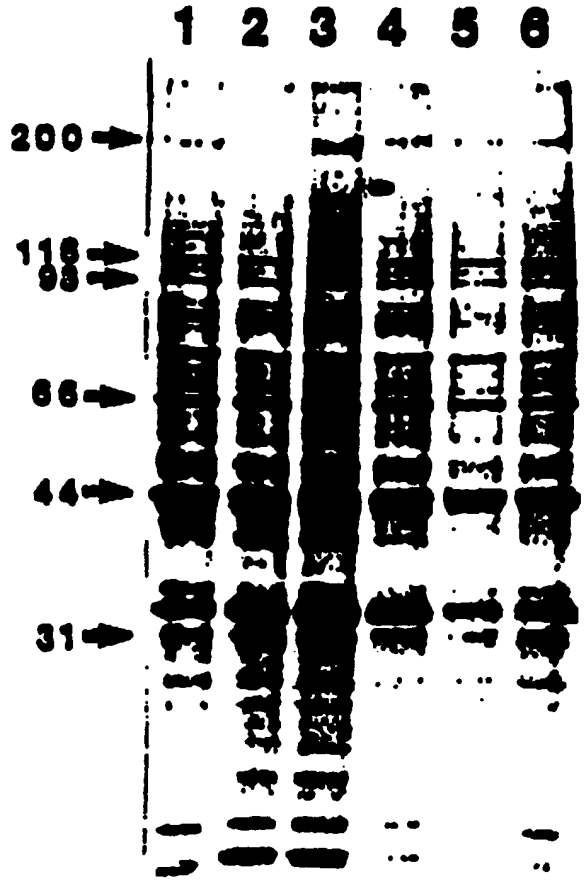

A
TNFα (pg/ml)
800
600
400
200
0
100
80
60
40
20
0
% OF ACTIVITY
.002
0.02
0.1
1.0
4
10
B
TNFα (pg/ml)
600
400
200
0
C
180
120
80
40
0
CONT
0
0.1
1
4
THALIDOMIDE (μg/ml)

Fig. 1


1 2 3 4 5 6
200
116
97
66
44
31

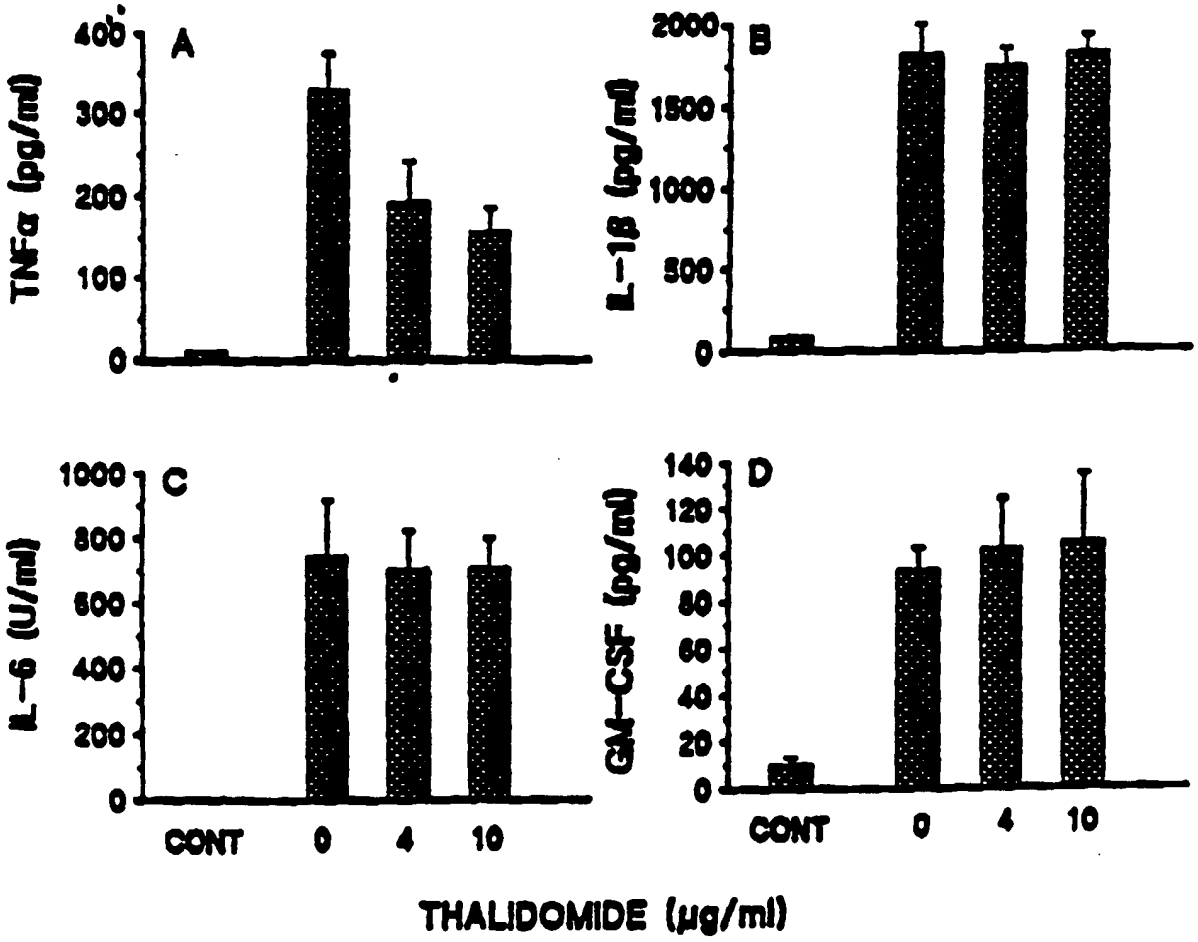

A
TNFα (pg/ml)
400
300
200
100
0
B
IL-1β (pg/ml)
2000
1500
1000
500
0
C
IL-6 (U/ml)
1000
800
600
400
200
0
D
GM-CSF (pg/ml)
140
120
100
80
60
40
20
0
CONT
0
4
10
THALIDOMIDE (μg/ml)

Fig. 3

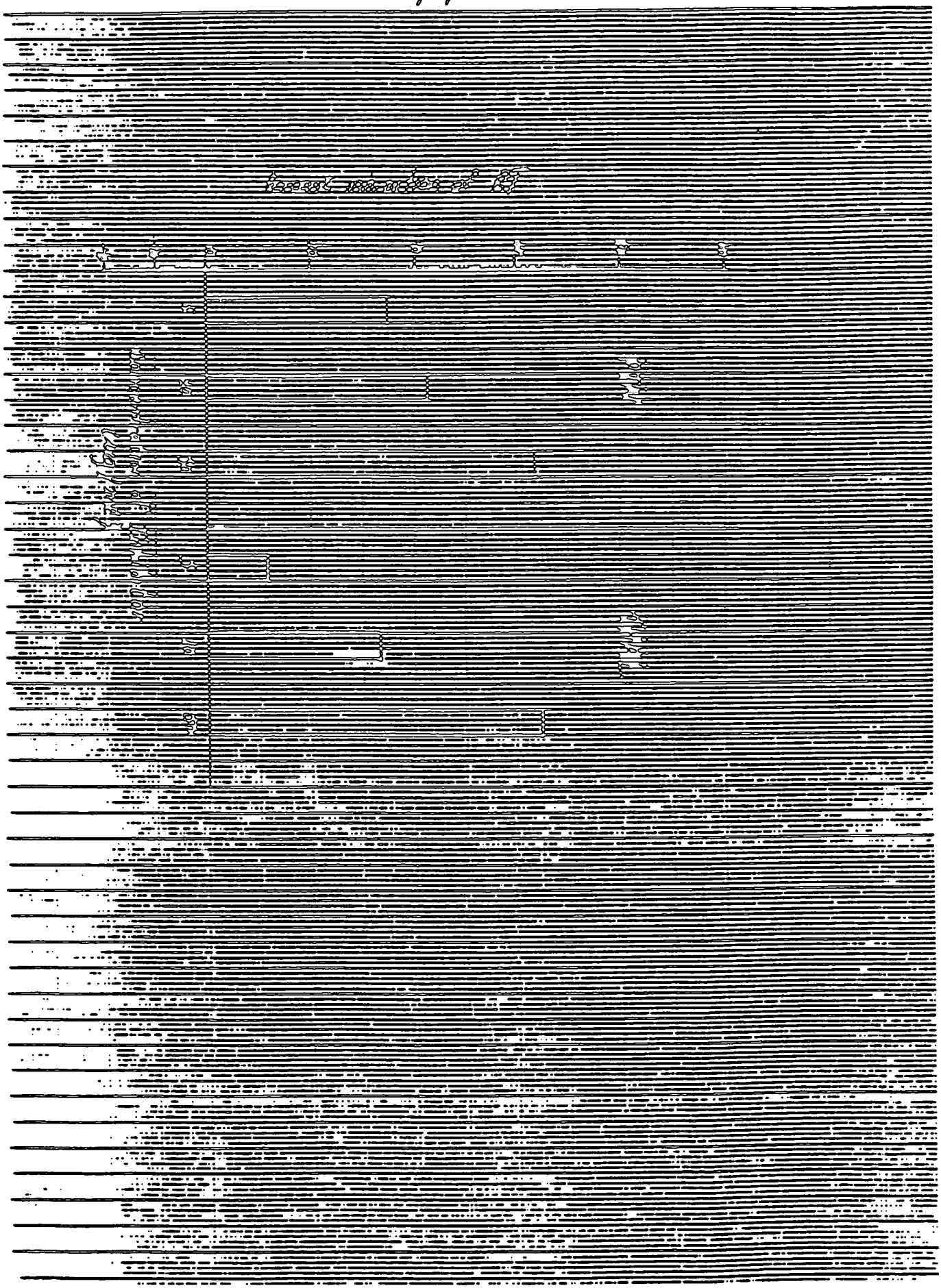


Figure 4

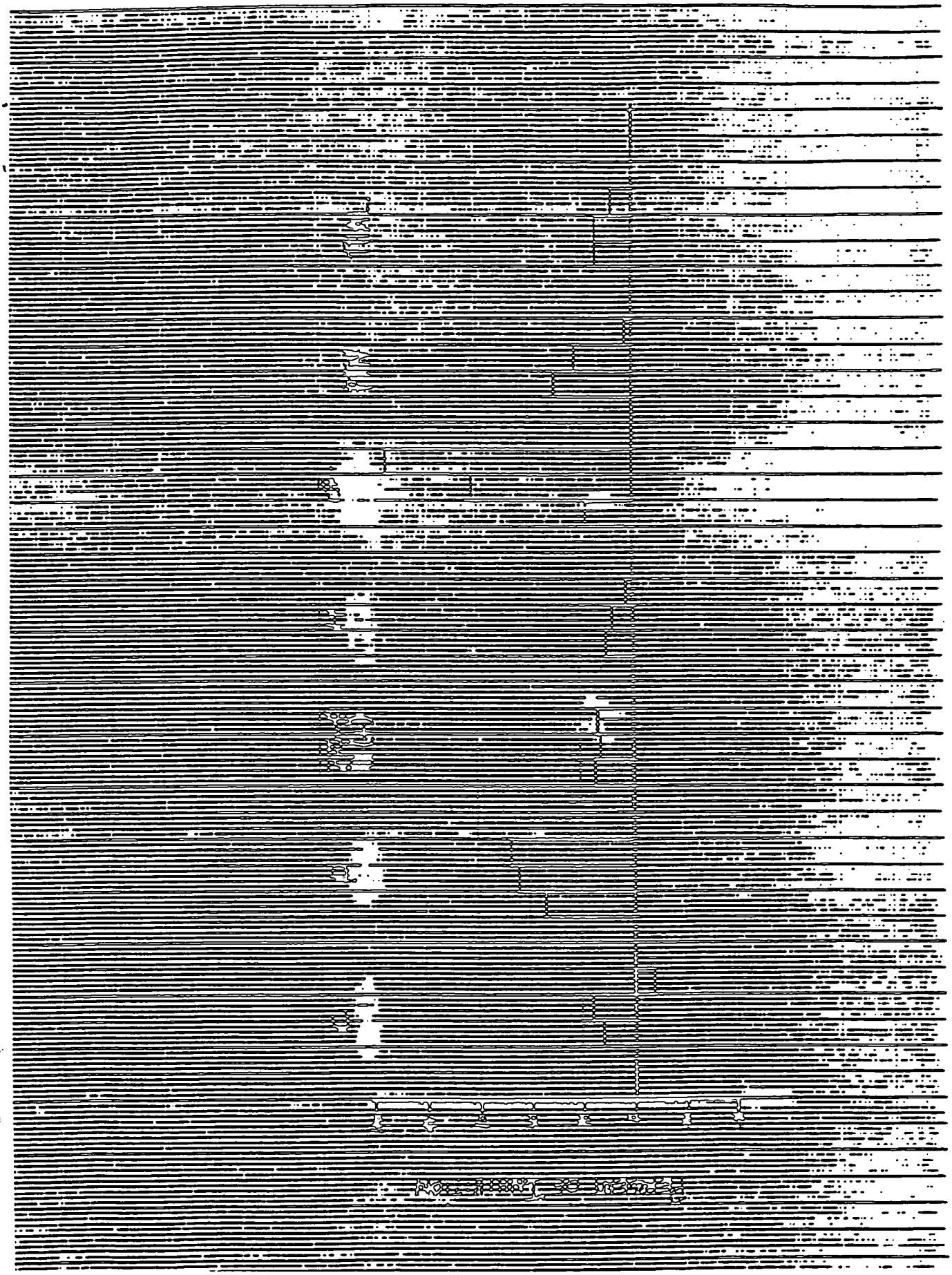


Figure 5

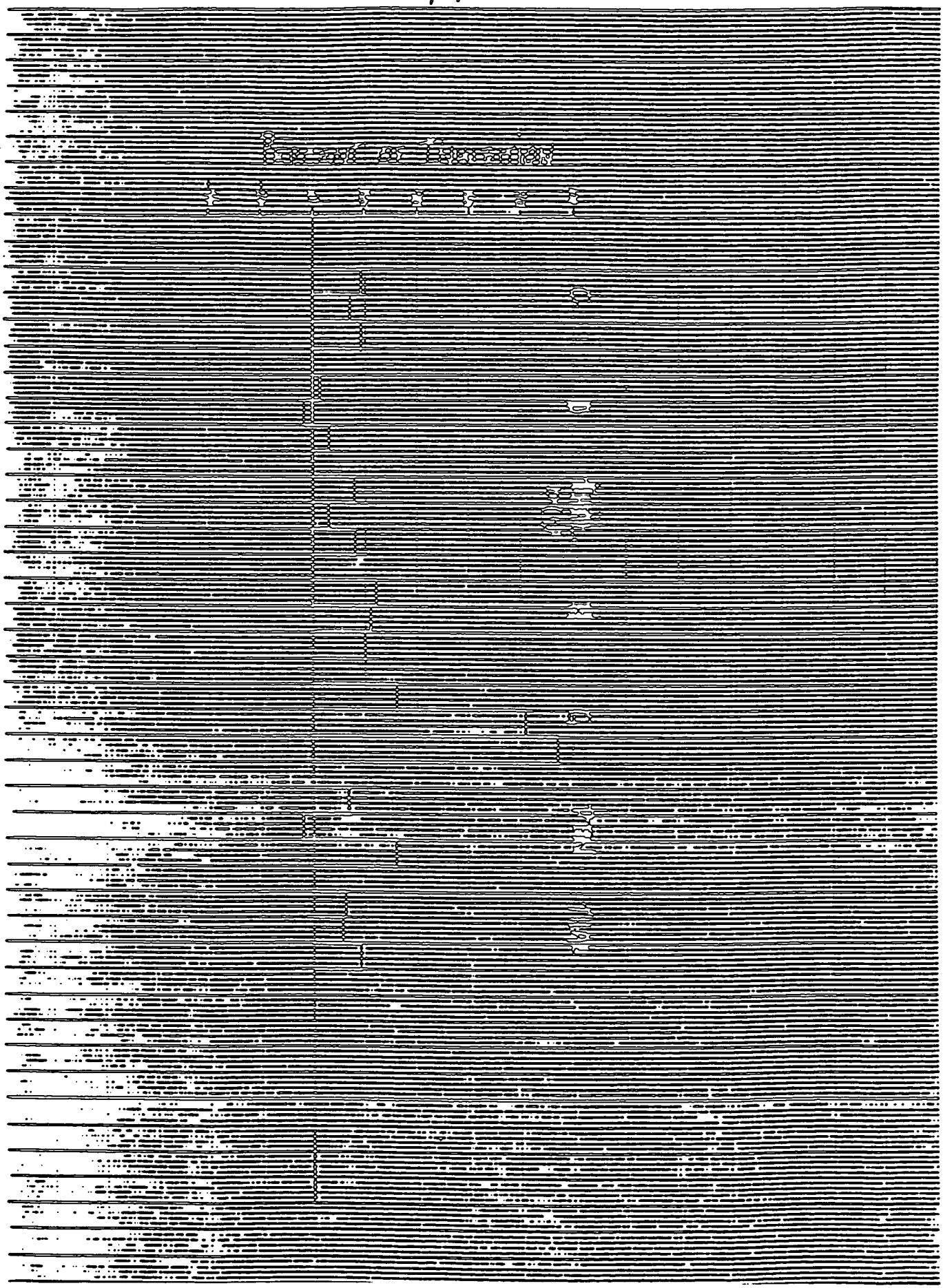


Figure 6

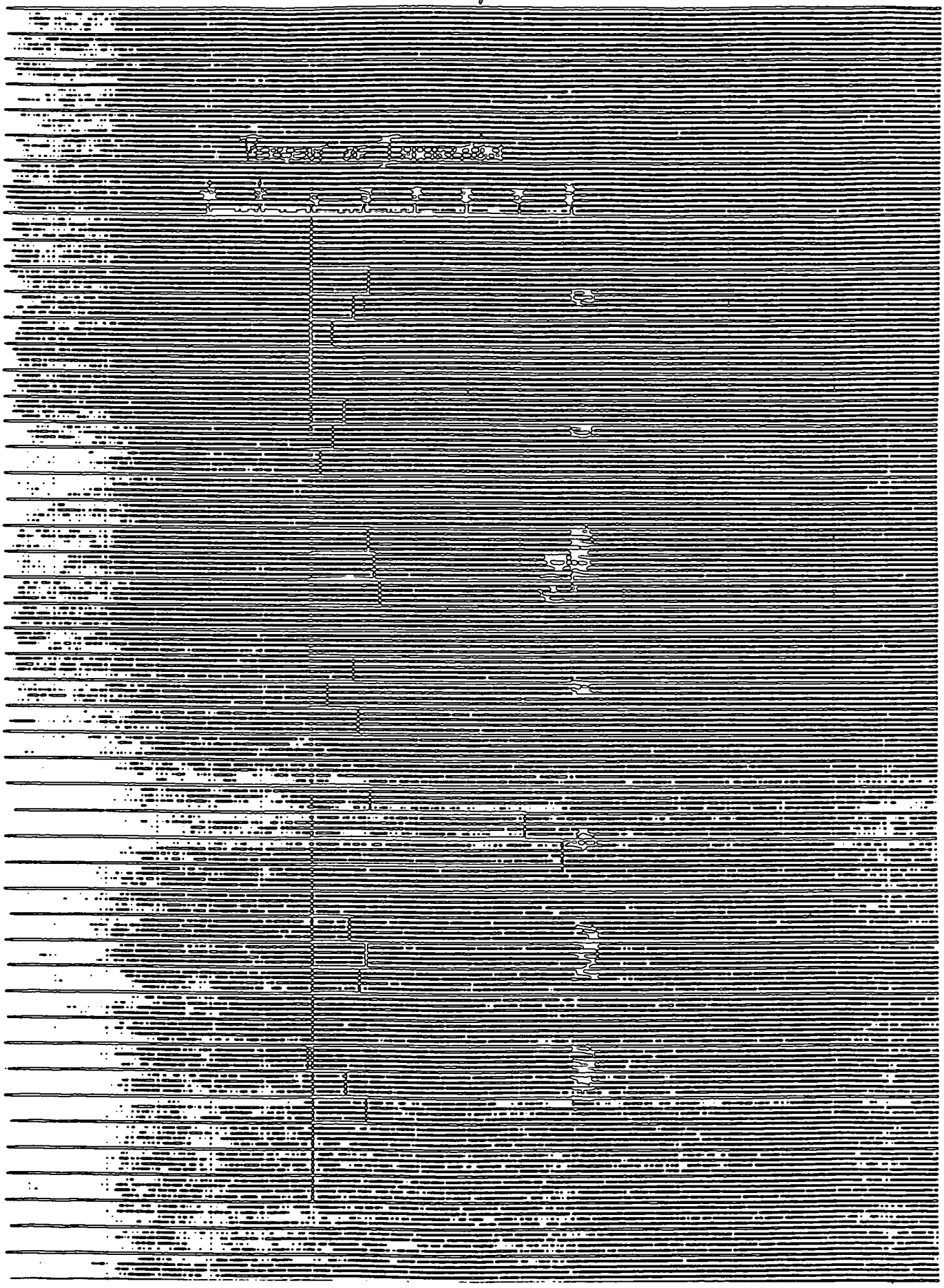


Figure 7

INTERNATI NAL SEARCH REPORT

International Application No. PCT/US92/01207

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 31/21, 445, 535

II. FIELDS SEARCHED

Minimum Documentation Searched †

Classification System	Classification Symbols
U.S. CL.	514 - 231.5 514-315, 514-317, 514-513

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched ‡

III. DOCUMENTS CONSIDERED TO BE RELEVANT §

Category *	Citation of Document, ‖ with indication, where appropriate, of the relevant passages ‖	Relevant to Claim No. ‖
A	CHEMICAL ABSTRACTS, VOLUME 109, NO. 188,557 H "Weight loss in obese mice persistently infected with lymphocytie choriomeningelis virus is not associated with elevated tumor necrosis factor/cach ectin activity in peritoneal macropages lathey et al. (1988).	1-16

* Special categories of cited documents: ‖

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 June 1992	09 JUL 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	S. Friedman

Form PCT/ISA/210 (second sheet) (Rev. 11-87)